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Genetic Studies of Human Apolipoproteins. X. The Effect of the Apolipoprotein E Polymorphism on Quantitative Levels of Lipoproteins in Nigerian Blacks

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Summary

Human apolipoprotein E exhibits genetic polymorphism in all populations examined to date. By isoelectric focusing and immunoblotting, three common alleles have been demonstrated in 365 unrelated Nigerian blacks. Furthermore, the APO E genetic polymorphism's effect on quantitative levels of lipids and lipoproteins has been determined. The respective frequencies of the *APO E*2, APO E*3,* and *APO E*4* alleles are .027, .677, and .296. The effect of APO E polymorphism is significant only on total cholesterol and low-density lipoprotein cholesterol. The average excesses of the *APO E*2* allele are to lower total cholesterol and low-density lipoprotein cholesterol by 9.19 mg/dl and 11.11 mg/dl, respectively. The average excesses of the *APO E*4* allele are to increase total cholesterol and low-density lipoprotein cholesterol by 5.64 mg/dl and 6.18 mg/dl, respectively. On the basis of the differences in (*a*) the distribution of APO E allele frequencies between the Nigerians and other populations and (*b*) dietary lipids, we propose a model that shows that lipid metabolism is influenced by the combined effects of the APO E polymorphism and environmental factors.

Introduction

Human apolipoprotein E (APO E) is an arginine-rich glycoprotein consisting of a single polypeptide chain composed of 299 amino acid residues with an estimated molecular weight of 33,000–39,000 daltons (Rall et al. 1981). APO E is a structural component of the chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and high-density lipoprotein (HDL) fractions of human plasma. APO E can serve as a ligand for two types of high-affinity receptors on liver cell plasma membranes, the APO E receptor (chylomicrons remnant receptor) and low-density lipoprotein (LDL) receptor (B/E receptor) (Innerarity and Mahley 1978; Pitas et al. 1981), which mediate the uptake of lipoproteins that contain APO E.

In man, the structural gene locus for APO E is polymorphic. Three common alleles—designated *APO E*2, APO E*3,* and *APO E*4*—determine six APO E phenotypes that can be distinguished by isoelectric focusing (Utermann et al. 1977, 1978; Havekes et al. 1987; Kamboh et al. 1988). The three alleles differ by an amino acid substitution at one or both of two sites (residues 112 and 158) (Rall et al. 1982). APO E 2 has cysteine at both sites, and APO E 4 has arginine at both sites, while APO E 3 has cysteine at site 112 and arginine at site 158. In population studies, the APO E allele frequencies are 0%–13% for *E*2*, 72%–89% for *E*3*, and 12%–18% for *E*4* (Davignon et al. 1988). These allele frequencies are significantly heterogeneous among populations. The APO E polymorphism has been found to influence plasma lipid metabolism. The relative frequency of the *APO E*2* allele is increased in patients

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with type III hyperlipoproteinemia (Utermann et al. 1977; 1978). The relative frequency of the *APO E*4* allele is increased in hypercholesterolemic individuals (Assmann et al. 1984; Leren et al. 1985). In all reported populations, the average effect of the *APO E*2* allele is to lower total cholesterol levels, while the average effect of the *APO E*4* allele is to raise total cholesterol levels.

Some progress has been made in the understanding of the structure, function, and genetics of APO E, but the physiological mechanism by which APO E polymorphism has its effect on cholesterol levels is not completely understood. The protein product of the *APO E*2* allele has a lower affinity for high-affinity lipoprotein receptors than does the common, *APO E*3,* allele (Mahley and Innerarity 1983; Hui et al. 1984). Individuals who are homozygous for the *APO E*2* allele do not efficiently clear chylomicron remnants because of impaired receptor interaction. The *APO E*4* allele product has a faster rate of clearance of these remnant lipoproteins from the plasma, as compared with *APO E*2* and *APO E*3* allele products (Gregg et al. 1986). These functional differences in APO E allele products explain, in part, the effect of the APO E polymorphism on lipid metabolism. Except for Caucasians, in whom the APO E locus has been studied extensively, there is a paucity of data on the distribution of APO E variation in world populations.

In the present study, we have used a rapid one-dimensional isoelectric focusing–immunoblotting technique (Kamboh et al. 1988) to determine the occurrence and frequency of genetic variation in APO E and have investigated the effect of the APO E polymorphism on quantitative variation in plasma lipids and lipoproteins in Nigerian blacks by using the measured-genotype approach (Sing and Davignon 1985; Boerwinkle et al. 1986).

Subjects and Methods

The study population consists of two groups. Group I (N = 173) are first-year medical students, nursing students, and midwifery students at the University of Benin, Benin City, Nigeria. Group II (N = 199) are a random sample of civil service workers and teachers from the Oroyo College Secondary School, Benin City, Nigeria. Of the total 372 samples, 7 were excluded because they could not be typed reliably for APO E polymorphism. The remaining sample of 365 unrelated subjects from the two groups consists of 193 males with an average ± SD height and weight of 168 ± 7.6 cm and 62.83 ± 9.31 kg, respectively. The 172 females have an average ± SD height and weight of 159 ± 6.2 cm and 58.10 ± 9.94 kg, respectively. Their age ranges are 17–54 years, with an average ± SD of 29.74 ± 9.5. A detailed description of the study population has been published elsewhere (Adams-Campbell et al. 1988; Bunker et al., submitted).

Prior to venipuncture, all subjects fasted about 16 h, and the blood samples were kept at room temperature for 2 h. The samples were centrifuged, and the serum was pipetted into glass tubes and was frozen at −47°C for 2 wk. A styrofoam ice chest with frozen cold packs was used to transport samples from Nigeria to Pittsburgh. In the nutrition laboratory at the University of Pittsburgh, Graduate School of Public Health, total serum cholesterol and triglycerides were measured manually using an enzymatic method (Bucolo and David 1973; Allan et al. 1974). Total HDL-C was determined by the heparin–manganese chloride precipitation method, whereas subfraction HDL3 cholesterol concentrations were measured after the precipitation of the HDL2 cholesterol subfraction by dextran sulfate (Gidez et al. 1977; Warwizk and Albers 1978). LDL cholesterol was calculated using the Friedewald equation (Friedewald et al. 1972). Aliquots of serum were transferred to the University of Pittsburgh Human Genetics Laboratory and were stored at −80°C prior to typing.

Sample treatment, isoelectric focusing, and immunoblotting were carried out essentially as described elsewhere (Kamboh et al. 1988), to screen APO E polymorphism.

The gene-counting method was used for the estimation of gene frequencies. Test of equality of genotype frequencies in two subgroups was performed by a contingency χ^2 test (Sokol and Rohlf 1981). For each lipid/lipoprotein variable, significant covariates were identified using a stepwise regression procedure. Effects of significant covariates were removed from the corresponding lipid/lipoprotein variable by estimating the multiple regression equation and subtracting the predicted value of the lipid/lipoprotein variable from its observed value. The skewness and kurtosis coefficients of each adjusted lipid/lipoprotein variable were then computed to examine departures from normality. Wherever appropriate, suitable transformations of the adjusted variables were performed to induce normality to the extent possible. For each adjusted variable—transformed or untransformed as the case may be—a two-way analysis of variance was performed. The analysis of variance model included gender and APO E phenotype as the main effects; an interaction term between the main effects was also included. These analy-

ses were performed using standard statistical packages. Average excesses of APO E alleles were computed whenever APO E phenotypic effect turned out to be significant. The formulas used for computing average excesses have been reported by Templeton (1987).

Results

For each of the two groups, gender differences in frequencies of APO E phenotypes were tested. The χ^2 values were nonsignificant at the 5% level in both groups. The genders were therefore pooled within each group. To test differences in phenotypic proportions between groups, a χ^2 test was performed. The value of this test statistic was also nonsignificant at the 5% level. The two groups were therefore pooled. In the pooled sample, the frequencies of APO E phenotypes 3-2, 3-3, 4-2, 4-3, and 4-4 are, respectively, 14, 176, 6, 128, and 41 (total sample size = 365). APO E allele frequencies estimated in the pooled sample are as follows: *APO E*2* = .027; *APO E*3* = .677; and *APO E*4* = .296. The observed genotype frequencies were found to be in agreement with Hardy-Weinberg expectations.

For each lipid/lipoprotein variable, a stepwise regression analysis was performed to identify which of the covariates among age, age^2, height, height^2, weight, and body mass index (BMI) was significant. For all variables, age and BMI were found to be the significant covariates; that is, after adjusting for the effects of age and BMI, the effects of the remaining covariates were nonsignificant at the 5% level. Multiple regression equations of lipid/lipoprotein variables on age and BMI were estimated, and the residual (observed − predicted) values, which are the lipid/lipoprotein values adjusted for the effects of significant covariates, were computed. The adjusted mean values of the serum lipid/lipoprotein variables are presented in table 1.

Normality of distributions of adjusted lipid/lipoprotein variables was examined. When there was a significant departure from normality, several transformations were tried to induce normality to the extent possible. It was found that, while the distributions of adjusted total cholesterol (TC) and LDL-cholesterol (LDL-C) levels were close to normal, the distributions of adjusted total HDL-cholesterol (HDL-C) and triglyceride (TG) levels were not. The logarithmic transformation was found to bring the distributions of adjusted total HDL-C and TG close to normal. The remaining analyses were therefore performed on untransformed values of adjusted TC and LDL-C values and on log-transformed values of adjusted total HDL-C and TG.

To test whether gender and/or APO E phenotype effects on adjusted (transformed or untransformed) lipid/lipoprotein levels were significant, two-way analyses of variance (with sex and APO E phenotypes as main effects and interaction between the two) were performed. For each variable the analysis of variance was performed both including and excluding individuals with the 3-2 and 4-2 phenotypes. This was done to avoid vagaries of small sample sizes, as the sample sizes for the 3-2 and 4-2 phenotypes were small. The results are presented in table 2. It is seen that neither of the main effects is significant for total HDL-C and HDL3-C. Only the effect of sex is significant for TG, while the effects of both sex and phenotype are significant for TC and LDL-C. Since the interaction effect is significant on total HDL-C, one-way analysis of variance was performed for total HDL-C by treating each sex separately to determine the phenotype effect within each sex. However, one-way analysis of variance revealed no significant effect of APO E phenotype on total HDL-C.

The average adjusted TC and LDL-C values among individuals carrying the *APO E*2* allele (i.e., the 3-2 and 4-2 phenotypes) are, respectively, 13.4 mg/dl lower

Table 1

Adjusted Mean[a] ± SE Values of Serum Lipid/Lipoprotein Variables among APO E Phenotypes in the Sample of 365 Nigerian Blacks

Lipid Variable	APO E Phenotype					Pooled Mean
	3-2	3-3	4-2	4-3	4-4	
TC (mg/dl)	153.92 ± 9.38	157.25 ± 2.30	149.50 ± 12.17	166.89 ± 2.86	171.23 ± 5.45	161.90 ± 1.84
Total HDL-C (mg/dl)	46.46 ± 3.89	47.23 ± .85	46.40 ± 5.95	46.13 ± 1.02	44.28 ± 1.75	46.47 ± .61
HDL3-C (mg/dl)	28.45 ± 1.70	30.52 ± .56	28.44 ± 2.73	29.40 ± .60	28.51 ± 1.20	29.79 ± .38
LDL-C (mg/dl)	91.80 ± 9.39	96.80 ± 2.07	87.60 ± 14.45	106.97 ± 2.59	112.60 ± 4.87	101.80 ± 1.67
TG (mg/dl)	77.82 ± 10.62	66.06 ± 2.07	75.17 ± 5.49	68.42 ± 2.31	72.37 ± 5.11	68.17 ± 1.65

[a] Unadjusted grand mean plus mean of the adjusted residual.

Table 2

Two-way Analysis of Variance For Testing Significances and the Effects of Sex, APO E Phenotype, and Interaction between Sex and Phenotype in Nigerian Blacks

	F-Ratio for Effect of					
	Sex		Phenotype		Sex × Phenotype	
Lipid Variable	Including 3-2 and 4-2 Phenotypes	Excluding 3-2 and 4-2 Phenotypes	Including 3-2 and 4-2 Phenotypes	Excluding 3-2 and 4-2 Phenotypes	Including 3-2 and 4-2 Phenotypes	Excluding 3-2 and 4-2 Phenotypes
TC	8.98* (1,355)	8.49* (1,339)	3.14* (4,355)	5.35* (2,339)	.79 (4,355)	1.11 (2,339)
Total HDL-C	.95 (1,355)	.98 (1,339)	.63 (4,355)	1.28 (2,339)	2.97* (4,355)	3.35* (2,339)
HDL3-C	.35 (1,355)	.20 (1,339)	1.03 (4,355)	1.71 (2,339)	1.12 (4,355)	1.47 (2,339)
LDL-C	9.72* (1,355)	9.00* (1,339)	4.64* (4,355)	7.73* (2,339)	1.22 (4,355)	.50 (2,339)
TG	9.43* (1,355)	8.68* (1,339)	1.06 (4,355)	1.11 (2,339)	.27 (4,355)	.41 (2,339)

Note.—Numbers in parentheses are df.
[a] Significant at 5% level.

and 15.8 mg/dl lower than the average adjusted levels of those not carrying the *APO E*2* allele (table 1). These differences are significant at the 5% level. On the other hand, the average adjusted TC and LDL-C levels among individuals carrying the *APO E*4* allele (i.e., the 4-2, 4-3, and 4-4 phenotypes) are, respectively, 7.0 mg/dl higher and 8.1 mg/dl higher than the average of those with no *APO E*4* allele. These differences are, however, not significant at the 5% level. The mean cholesterol and LDL-C levels are the lowest among individuals of the 4-2 phenotype, and the average TC and LDL-C values are highest among individuals of the 4-4 phenotype.

The average excesses of different APO E alleles on levels of lipid variables were also estimated for TC and LDL-C. The values are presented in table 3. The effects of the APO E allele on lipid variables are generally in agreement with those reported by others (e.g., Davignon et al. 1988). The effect of the *APO E*2* allele is to lower the cholesterol and to raise the triglycerides, and the effect of the *APO E*4* allele is the opposite.

Discussion

Early studies on the distribution of APO E genetic polymorphism in Caucasians suggested that APO E allele frequencies are more or less homogenous and are independent of ethnic and cultural background (Sing and Davignon 1985). Further studies—with more distantly related populations, such as Amerindians and Japanese (Asakawa et al. 1985), Chinese (Wang 1986), Indians, and Malays from Singapore (Utermann 1987)—showed there may be a pattern of heterogeneity of allele frequencies among different ethnic groups. To the best of our knowledge there is no reported study in black populations which examines the effect of APO E polymorphism on quantitative lipid levels. In the present study we have determined that the APO E locus is polymorphic in Nigerian blacks. The *APO E*3* is the most common allele, followed by the *APO E*4* and *APO E*2* alleles. The distribution pattern of APO E allele frequencies in Nigerian blacks is significantly different than those in all other population groups reported previously elsewhere (e.g., see Davignon et al. 1988). The Nigerian black are characterized by having both a high frequency of the *APO E*4* allele and comparatively low frequencies of the *APO E*2* and *APO E*3* alleles. The effect of APO E polymorphism on lipid and lipoprotein levels was estimated by the measured-genotype approach. In the Nigerian population, the effects of the APO E polymorphism are in general agreement with those reported in other world populations (Davignon et al. 1988). The effect of the *APO E*2* allele is to lower cholesterol levels and to raise triglyceride levels, and the effect of the *APO E*4* allele is to raise cholesterol.

Utermann (1987) and Boerwinkle and Utermann (1988) have proposed a model by which the APO E

Table 3

Average Excesses of APO E Alleles on Total and LDL Cholesterol Levels in Nigerian Blacks

	Average Excess of		
Lipid Variable	*APO E*2*	*APO E*3*	*APO E*4*
TC (mg/dl)	−9.19	−2.10	5.64
LDL-C (mg/dl)	−11.11	−2.25	6.18

polymorphism affects plasma LDL-C levels and, therefore, total plasma cholesterol. In this model the E 2 isoprotein has a low binding affinity for the hepatic APO E receptor, and this leads to an accumulation of chylomicron remnants and HDL particles containing APO E in the plasma. This is reflected in the higher mean APO E levels seen in APO E 2-2 individuals. Less exogenous cholesterol enters the liver by the APO E receptor–mediated pathway, and there is an increased synthesis of B/E receptors, which enhances the uptake of LDL-C. This results in lowering LDL-C and TC levels in the plasma. The E 4 isoprotein has a higher turnover rate (relative to the E 3 isoprotein), and E 4–containing particles are rapidly internalized by the liver (Gregg et al. 1986). This is reflected in lower plasma APO E levels in individuals carrying the E 4 isoprotein. Enhanced uptake leads to higher intracellular cholesterol levels and to reduced synthesis of B/E receptors and accumulating plasma LDL-C. Competition experiments in patients with defective B/E receptors suggest that each APO E–containing HDL particle interacts with at least four sites on the B/E receptor (Innerarity et al. 1980, 1981). Thus, in a heterozygous individual, APO E–containing particles will contain a mixture of E 2 and E 4 isoprotein species. Under a strict gene dosage model, one would expect that the cholesterol levels in the 4-2 heterozygote would be intermediate between the E 2 and E 4 homozygotes. In all studies the 4-2 phenotype is associated with cholesterol levels higher than the predicted mean as based on levels in the two homozygotes. Thus, while expression of the *APO E*2* and *APO E*4* alleles is codominant at the gene level, the *APO E*4* allele exhibits a degree of dominance at the phenotype level. These observations must be viewed with caution because of the small number of APO E 4-2 heterozygotes observed in most studies.

In all studies the mean plasma cholesterol level in homozygous, E 3-3, individuals is statistically indistinguishable from the overall population mean, as is expected on the basis of the relative frequency of the three alleles in populations. The *APO E*4* allele is associated with higher mean cholesterol levels in populations, and one might expect that a higher *APO E*4* allele frequency would be associated with a higher mean cholesterol value, as was observed by Ehnholm et al. (1986) in Finns. To date, Nigerian blacks have the highest observed frequency of the *APO E*4* allele in world populations, but their adjusted mean cholesterol level is among the lowest reported in studies of the cholesterol/APO E relationship. This is probably due to a diet that is low in animal fat and high in saturated fat because of the consumption of palm oil. Despite this major dietary difference, the average effect of the *APO E*4* allele is to increase cholesterol levels. This supports the idea that the allelic effects at the APO E locus are the same in all populations but that the magnitude of these effects are modulated by diet. The one exception to the pattern of allelic effects comes from the report by Utermann (1987) that, among Chinese, Indians, and Malays living in Singapore, the average effect of the *APO E*4* allele was not to increase plasma cholesterol levels. While this exception to the general pattern may be due to dietary factors, the present work suggests the possibility of genetic heterogeneity within the *E*4* class of alleles. As studies of the APO E polymorphism have extended beyond mixed European and U.S. Caucasians, the apparent homogeneity of allele frequencies at this locus has not been confirmed, and population differences may serve as a tool to investigate the APO E–environment interactions in lipid metabolism.

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